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TRANSACTIONS

Oryzanin "Antineuritic Vitamin". V.

On the Activity of Oryzanin Hydrochloride

By Sator OHDAKE and Teikichi YAMAGISHI

(Agricultural Chemical Laboratory, Faculty of Agriculture, Tokyo Imperial University, Komaba, Tokyo.)

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In the previous paper,[1] the authors have reported that 0.001 mg. of oryzanin hydrochloride is the minimum for maintaining the normal growth of young albino rats and 0.001～0.0015 mg. are equivalent to the standard unit. The present experiment has been carried out to determine the B_1 activity of the same hydrochloride by the "curative test" for pigeons.

Experimental

(A) The curative "day dose" for pigeons;

The curative "day dose" was tested by the method of Kinnersley and Peters.[2] When pigeons weighing about 300 g. showed the severe symptoms (head retraction) of B_1 deficiency on a diet of polished rice, oryzanin hydrochloride was given in an aqueous solution either orally or by a subcutaneous injection and the curative activity was compared with that of the standard product. The results are summarised in the following table;

Table 1, B_1 activity by the "day dose" test;

Pigeon No.	Body weight g.	Days to B_1-deficiency	Body-weight at B_1-deficiency g.	Dose mg.	Days of cure	"Day dose" mg.
(I) The standard adsorption product*(orally)						
1) 721	312	15	234	150	9	16.7
2) 724	312	28	171	100	8	12.5
3) 745	307	16	193	100	5	20.0
4) 740 (i)	320	17	227	100	7	14.3

5) 740 (ii)	320	24	212	100	5	20.0
6) 742	315	22	166	100	6	16.7
7) 758	320	13	207	100	5	20.0
8) 726	333	32	195	80	3	26.7
9) 739	348	21	231	50	6	8.3
10) 755	345	23	207	50	4	12.5
11) 756	340	24	184	50	3	16.7
12) 854	305	23	235	50	7	7.2
Average day dose						16.0

(II) Oryzanin hydrochloride from rice polishings (orally);

1) 720 (i)	322	12	239	0.015	7	0.0021
2) 720 (ii)	322	19	193	0.010	4	0.0025
3) 722	342	14	243	0.010	4	0.0025
4) 721 (i)	334	24	190	0.010	4	0.0025
5) 727 (ii)	334	28	170	0.010	6	0.0017
6) 732	341	22	257	0.010	7	0.0014
7) 736	305	19	215	0.010	4	0.0025
8) 741 (i)	330	22	212	0.010	7	0.0014
9) 729 (i)	334	17	263	0.009	2	0.0045
10) 720 (ii)	334	19	243	0.009	3	0.0030
11) 752	318	27	227	0.008	4	0.0020
12) 741 (ii)	330	29	200	0.008	4	0.0020
Average day dose						0.0023

(III) Oryzanin hydrochloride from rice polishings (by injection);

1) 333	342	22	191	0.012	4	0.0030
2) 310 (i)	321	24	202	0.010	3	0.0033
3) 310 (ii)	321	27	196	0.010	3	0.0033
4) 331	289	22	177	0.010	5	0.0020
5) 332	285	18	179	0.010	4	0.0025
6) 334	274	18	185	0.010	5	0.0020
7) 311	328	34	181	0.008	3	0.0027
8) 312	302	25	196	0.008	5	0.0016
9) 313 (i)	304	32	172	0.008	3	0.0027
10) 312 (ii)	304	35	176	0.008	3	0.0027
11) 324 (i)	342	26	205	0.008	4	0.0020
12) 324 (ii)	342	30	194	0.008	4	0.0020
13) 326	317	27	205	0.008	3	0.0027
14) 336	310	23	209	0.008	6	0.0013
15) 330	291	28	184	0.005	3	0.0017
Average day dose						0.0024

(IV) Oryzanin hydrochloride from yeast (by injection);

1) 455	300	27	184	0.012	6	0.0020
2) 453	282	30	205	0.010	3	0.0033
3) 451	307	22	220	0.008	2	0.0040
4) 487	320	32	202	0.008	3	0.0027
5) 488	325	18	212	0.008	3	0.0027
6) 490 (i)	295	24	224	0.008	4	0.0020
7) 490 (ii)	295	28	227	0.008	4	0.0020
8) 491	295	32	209	0.008	3	0.0027
9) 493 (i)	307	16	206	0.008	5	0.0016
10) 493 (ii)	307	21	190	0.008	4	0.0020
11) 506	325	21	210	0.008	3	0.0027
12) 526	298	23	208	0.006	5	0.0012
Avearge day dose						0.0024

(V) "Injectio Oryzanin Fortior"** of Sankyo & Co. (by injection);

1) 750 (i)	312	13	234	0.020 cc (10γ.)	4	0.0050 (2.5γ.)
2) 759	334	14	239	0.020 cc (10″)	5	0.0040 (2.0″)
3) 781	319	25	177	0.020 cc (10″)	4	0.0050 (2.5″)
4) 782	292	23	182	0.020 cc (10″)	3	0.0066 (3.3″)
5) 750 (ii)	312	17	216	0.016 cc (8″)	4	0.0040 (2.0″)
6) 768	302	16	242	0.016 cc (8″)	4	0.0040 (2.0″)
7) 773	309	15	242	0.016 cc (8″)	3	0.0054 (2.7″)
8) 784	329	29	191	0.016 cc (8″)	5	0.0032 (1.6″)
Average day dose						0.0046 (2.3γ.)

* The standard product distributed by the National Institute for Medical Research, London.

** "Injectio Oryzanin Fortior" of Sankyo & Co. is the vitamin B_1 preparation contained in 1 cc. 0.5 mg. of B_1 crystals isolated from rice polishings.

From the above results, it can be observed that 0.0024 mg. of the crystalline hydrochloride possess the same activity with 16 mg. of the standard adsorption product, *i. e.* 1.5 γ. of the hydrochloride are equivalent to the standard unit. "Injectio Oryzanin Fortior" gave also the same result.

(B) The curative test for pigeons;

The minimum curative daily dose of the crystalline hydrochloride of oryzanin was tested on pigeons, fed on polished rice as well as on an artificial diet, deficient in Vitamin B_1 and the results were compared with that of the standard product.

The standard adsorption product;

(1) Pigeons weighing about 300 g., suffering from B_1-deficiency on a diet of polished rice have been used for the tests. When administrated daily with 20 mg. of the standard product orally for a week, the disease was thereby improved after few hours and completely cured in 1～2 days. Though the body weight declined gradually, they were protected perfectly from the symptoms. Better results were obtained with a daily dose of 30, 50, and 80 mg. respectively while 10 mg. were found to be insufficient. (cf. Chart. 1 & 2)

(2) When pigeons were fed on an artificial diet, deficient in vitamin B_1, consisting of 60% of purified starch, 20% of purified casein, 15% of arachis oil, 5% of McCollum's salts mixture (No. 185) and supplemented daily with 3 drops of codliver oil and 0.4 g of autoclaved yeast, they developed severe symptoms of B_1-deficiency usually in 3～4 weeks. By giving daily 20 mg. of the standard product orally, they were cured completely in one day and protected perfectly from B_1-deficiency. (cf. Chart. 3)

Crystals of oryzanin hydrochlororide;

(3) Pigeons suffering from B_1-deficiency on a diet of polished rice, when administrated daily with 0.003 mg. of oryzanin hydrochloride per os, were cured in one day and protected perfectly from the disease though their weight declined gradually. (Chart. 4)

(4) Pigeons, fed on polished rice and suffering from B_1-deficiency, were

cured perfectly in one day by the subcutaneous injection of 0.003 mg. of oryzanin hydrochloride. (Chart. 5)

(5) Pigeons suffering from B_1-deficiency on the artificial diet as mentioned above, were perfectly cured in one day by the administration of 0.003 mg. of the hydrochloride and increased in weight. (Chart 9)

It is observed that 0.003 mg. of the hydrochloride have the same activity with 20 mg. of the standard adsorption product, *i. e.* 0.0015 mg. are equivalent to the standard unit.

(6) By the subcutaneous injection in a daily dose of 0.002 mg. of the hydrochloride, pigeons cured completely in one day though the body weight declined gradually toward the end of the experiment. A daily dose of 0.003 mg. gave a satisfactory result. (Chart. 7 & 8)

Generally, in the curative test, the injection method used to give a better result, especially, when the pigeons were fed on an artificial diet.

"Injectio Oryzanin Fortior";

(7) "Injectio Oryzanin Fortior" of Sankyo & Co. is the vitamin B_1 preparation containing in 1 cc. 0.5 mg. of B_1-crystals isolated from rice polishings. The activity of this preparation was tested by injection in daily doses of 0.002 cc. (=0.001 mg.), 0.004 cc. (=0.002), 0.006 cc. (=0.003 mg.), 0.01 cc. (=0.005 mg.), and 0.02 cc. (=0.01 mg. of the crystalline hydrochloride) respectively, on pigeons suffering from B_1 deficiency by feeding on the artificial diet as mentioned above and 0.006 cc. (=0.003 mg.) were proved to have the same activity with 0.003 mg. of the crystals of oryzanin hydrochloride or with 20 mg. of the standard product. Thus 0.003 cc. (=0.0015 mg. of crystals) of the preparation are equivalent to the standard unit. (Chart. 9 & 10)

(C) The protective test for pigeons;

(3) The pigeons, fed on an artificial diet deficient in vitamin B_1 and administrated daily with 0.005, 0.01, 0.02, and 0.04 mg. of the crystalline oryzanin hydrochloride respectively, from the begining of the experiment, remaind in perfect health for 50 days. When the administration of the hydrochloride was ceased, they developed the symptoms of B_1-deficiency in 7～10 days.

Table 2. B_1-activity by the curative test for pigeons (average).

Materials	Number of Pigeons	Body weight g	Days, to B_1-deficiency	Body weight, at B_1-deficiency. g	Cured in, hrs.	Body weight, last 7 th. day. g	Days, to next B_1-deficiency	Body weight at next B_1-deficiency g
The standard adsorption product, by oral administration; (1) on polished rice;								
10.0 mg	3	332	28	230	insufficient, died after 2.4 days			
20.0 "	3	321	19	204	2 days	198	3	172
30.0 "	3	316	25	184	1 "	188	4	189*
50.0 "	3	334	27	216	1/2 "	215	7	188*
80.0 "	2	319	18	225	3 hrs	259	10	211

(2) on artificial diet;								
10.0 mg	4	329	25	242	insufficient, died after 6 days			
20.0 "	2	324	31	225	1 days	251	7	232
30.0 "	2	313	36	242	1 "	253	7	242
Oryzanin hydrochloride, on polished rice;								
(3) by oral administration;								
0.003 mg	2	305	24	192	1 days	173	4	172*
0.005 "	1	312	25	210	1/2 "	206	6	187*
0.008 "	1	321	22	235	3 hrs	257	9	212
(4) by injection;								
0.002 mg	3	306	25	185	insufficient, died after 1 day.			
0.003 "	3	297	17	203	4 hrs:	203	3	194
0.004 "	1	319	19	202	3 "	202	3	177
0.005 "	1	315	23	184	1-2 "	194	3	182*
0.008 "	1	274	21	169	1 "	168	6	150
0.010 "	1	285	21	174	1 "	177	6	152
Oryzanin hydrochloride, on artificial diet;								
(5) by oral administration;								
0.002 mg	3	317	37	232	2 days	223	2	214
0.003 "	3	342	36	262	1 "	268	5	245
0.004 "	1	303	15	235	1 "	235	4	203
(6) by injection;								
0.001 mg	3	320	22	240	insufficient, died after 6 days.			
0.002 "	3	321	26	254	1 day	235	3	224*
0.003 "	2	311	29	238	1 "	263	6	221
0.004 "	1	306	—	271	4 hrs	287	4	258
0.005 "	5	320	24	210	3 "	223	4	207
0.008 "	1	291	26	174	2 "	182	4	160
0.010 "	4	308	24	216	1 "	240	8	204
"Injectio Oryzanin Fortior", on artificial diet;								
(7) by injection;								
0.001 mg	3	321	34	240	1 day	230	3	220
0.002 "	3	321	34	233	1 "	238	4	222
0.003 "	3	338	30	244	1/2"	247	3	232
0.005 "	2	—	—	223	2 hrs	246	7	211
0.010 "	4	—	—	235	1 "	270	—	—

*)……died

Summary

The above results are summarised in the following table;

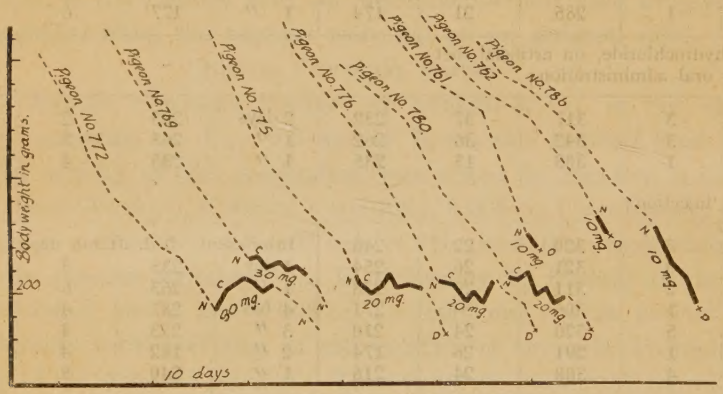
Materials	Rats growth dose mg.	Pigeons "day dose" mg.	Pigeons curative dose mg.
The standard adsorption product	10.0	15.0	20.0
Oryzanin hydrochloride, isolated from rice polishings as well as from yeast	0.001～0 0015	0.0023～0.0025	0.003
"Injectio Oryzanin Fortior" of Sankyo & Co.	0.002～0.003 cc (=0.001～0.0015 mg. crystals)	0.0046 cc (=0.0023 mg. crystals)	0.006 cc (=0.003 mg. crystals)

From the above value of B_1 activity, it can be concluded that 0.0015 mg. of the crystalline oryzanin hydrochloride isolated from rice polishings as well as from yeast are equivalent to the standard unit.

The authors wish to express their sincere thanks to Prof. Dr. U. Suzuki for his kind advice throughout the work and to Sankyo & Co. for the supply of the test material. They are also indebted to Messrs. M. Kamada and T. Hayakawa for their kind help in the curative test. (Tokyo. June 10 1935.)

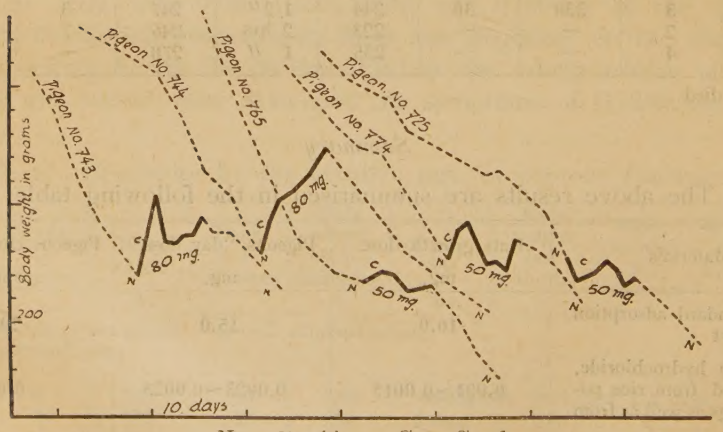
Literature

(1) S. Ohdake & T. Yamagishi; Bull. Agric. Chem. Soc. Japan. **11** (1935) 51~61.
(2) Kinnersley & Peters; Bioch. J. **19** (1925) 820.



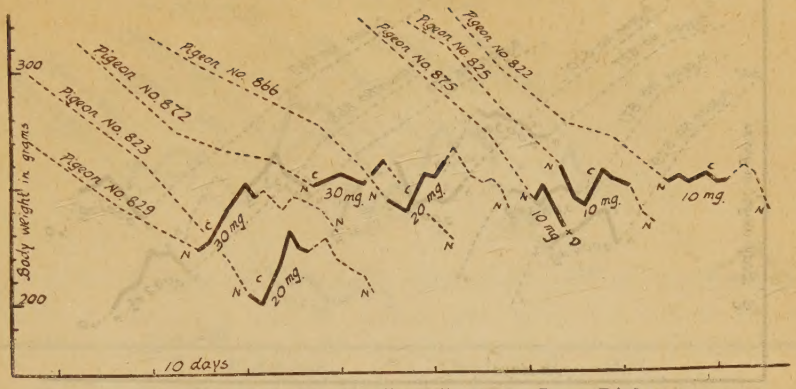
N……B_1-deficiency. C……Cured. D……Died.

Chart. 1 The curative test of the standard product for pigeons fed on a diet of polished rice. (10~30 mg)



N……Neuritis. C……Cured.

Chart. 2 The curative test of the standard product for pigeons fed on a diet of polished rice. (50~80 mg)

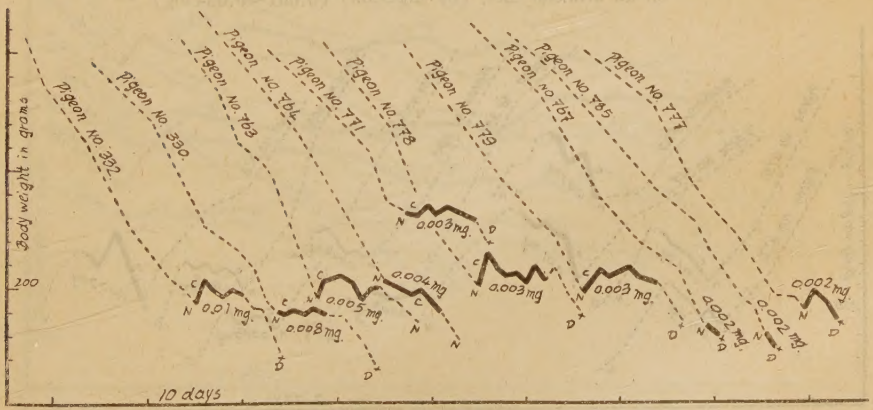


N……B_1-*deficiency*. *C*……*Cured*. *D*……*Died*.

Chart. 3 The curative test of the standard product for pigeons, fed on an artificial diet. (per os)

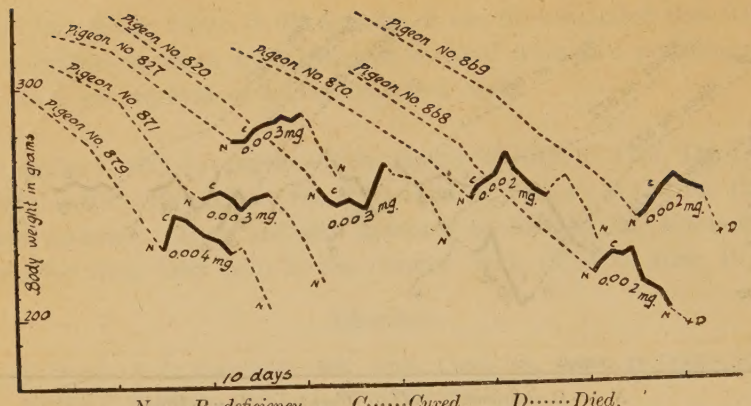
N……*Neuritis*. *C*……*Cured*.

Chart. 4 The curative test of oryzanin hydrochloride for pigeons, fed on a diet of polished rice. (per os)



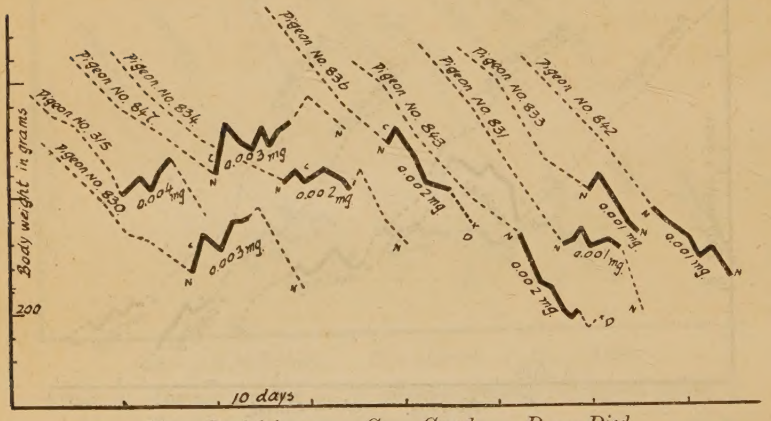
N……B_1-*deficiency*. *C*……*Cured*. *D*……*Died*.

Chart. 5 The curative test of oryzanin hydrochloride for pigeons, fed on a diet of polished rice. (by injection)



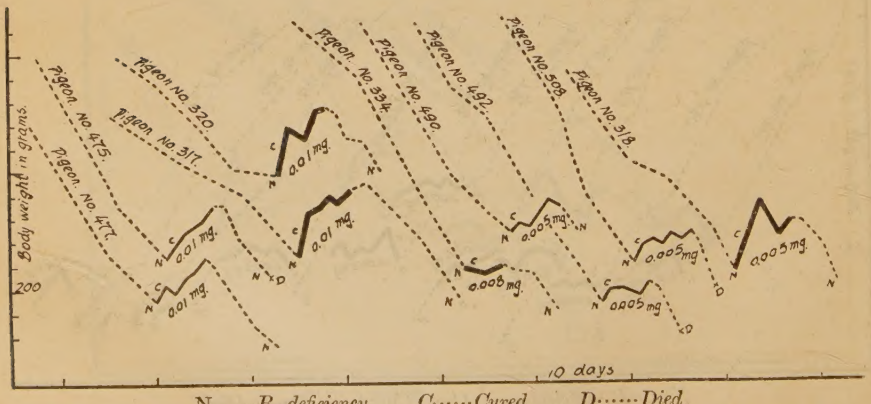
N……B_1-*deficiency*. *C*……*Cured*. *D*……*Died*.

Chart. 6 The curative test of oryzanin hydrochloride for pigeons, fed on an artificial diet. (per os)



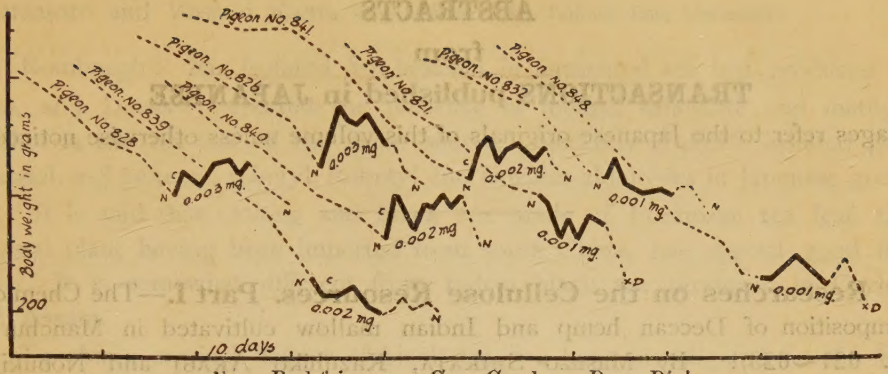
N……B_1-*deficiency*. *C*……*Cured*. *D*……*Died*.

Chart. 7 The curative test of oryzanin hydrochloride for pigeons, fed on an artificial diet. (by injection) (0.001～0.004 mg)



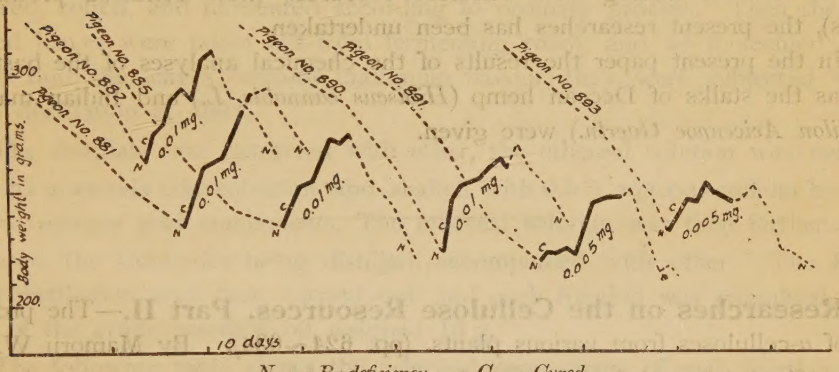
N……B_1-*deficiency*. *C*……*Cured*. *D*……*Died*.

Chart. 8 The curative test of oryzanin hydrochloride for pigeons, fed on an artificial diet. (by injection) (0.005～0.01 mg)



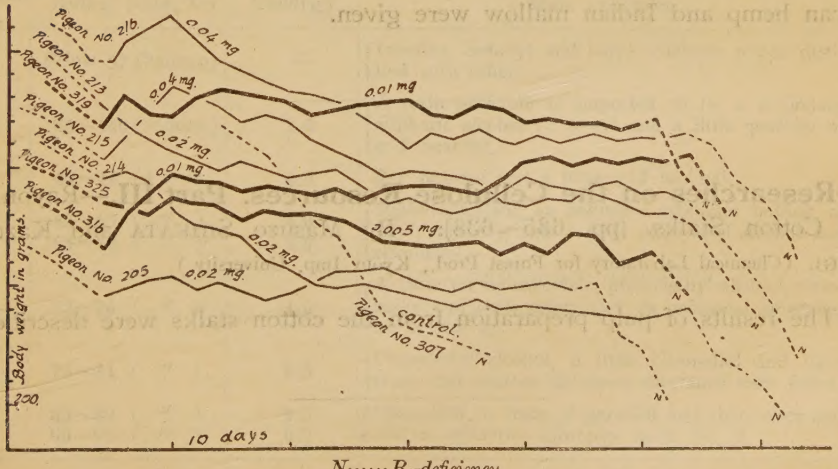
N……B_1-deficiency. C……Cured. D……Died.

Chart. 9 The pigeons curative test of "Injectio Oryzanin Fortior" for pigeons, fed on an artificial diet (by injection) (0.001~0.003 mg)



N……B_1-deficiency. C……Cured.

Chart. 10 The curative test of "Injectio Oryzanin Fortior" for pigeons, fed on an artificial diet (by injection) (0.005~0.01 mg)



N……B_1-deficiency.

Chart. 11 The protective test of oryzanin hydrochloride for pigeon, fed on an artificial diet, (per os) (0.005~0.04 mg)

ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

Researches on the Cellulose Resources. Part I.—The Chemical Composition of Deccan hemp and Indian mallow cultivated in Manchuria. (pp. 621～623): By Masuzo SHIKATA, Kazuhiko AKAGI and Nobukiyo URANO. (Chemical Laboratory for Forest Prod., Kyoto Imp. University.)

In view of finding new cellulose resources (new cellulose producing plants), the present researches has been undertaken.

In the present paper the results of the chemical analyses of the basts as well as the stalks of Deccan hemp (*Hibiscus cannabis L.*) and Indian mallow (*Abutilon Avicennoe Gaertn.*) were given.

Researches on the Cellulose Resources. Part II.—The properties of α-celluloses from various plants. (pp. 624～634): By Mamoru WATANABE. (Chemical Research Institute, Kyoto Imp. University.)

The comparison of the α-celluloses of cotten seed hairs, cotten bast fibre, Deccan hemp and Indian mallow were given.

Researches on the Cellulose Resources. Part III.—Rayon Pulp from Cotton Stalks. (pp. 635～638): By Masuzo SHIKATA and Kazuhiko AKAGI. (Chemical Laboratory for Forest Prod., Kyoto Imp. University.)

The results of pulp preparation from the cotton stalks were described.

Study on the Essential Oil of Black Tea. Part I.—The Essential Oil of Fermented Formosan Tea Leaf. (Vol. 10, pp. 661～667): By Ryo

YAMAMOTO and Yosiaki KATO. (Agr. Chem. Lab., Taihoku Imp. University)

Romburgh[1] has isolated β-γ hexenol in fermented tea leaf produced in Java, and Deuss[2] reported the presence of methyl salicylate and methyl-alcohol in the tea leaf of the same source. Recently Takei[3] isolated β-γ-hexenol, α-β-hexenal, n-butyl, isobutyl and isovaler aldehydes in Japanese green tea. It is said that Oolong and black tea made of Formosan tea leaf, the original plant having been imported from south China, has special good flavour. It is somewhat different from Indian black tea produced in Ceylon and Assam.

To obtain original flavour of black tea, we have investigated on the fermented tea leaves, just before drying. Four hundreds kg tea leaves of Seisin-Oolong variety which is known as a common variety in Formosa, were withered, rolled, and fermented according to ordinary process. Then the fermented leaves were taken out from fermenting room, and as it seemed that at this time the leaves contains maximum flavour, they were subjected to a steam distillation at 100°.

The distillate was extracted with ether, the ethereal solution was evaporated to a certain concentration, and shaken with 0.5% aqueous sodium hydroxide to remove acid components. The ethereal solution was then further concentrated, the aldehydes being distilled, accompanied with ether. The fractional distillation was then carried out and each fraction was examined. A yield of the crude essential oil weighed 13 g.

The following table shows the principal components of each fraction.

Distillat No.	Boiling point(°C)	Yield(g)	Main principles
1	Below 37 (760mm)	—	Isovaler, isobutyl and butyl aldehyde which distilled with ether.
2	84~140 (760mm)	1.0	A main principle is suspected to be a secondary aliphatic alcohol (c 4~6) and a little quantity of α-β hexenal.
3	40~44 (4 mm)	4.0	β-γ hexenol and a little α-β hexenal.
4	60~63 (〃)	4.0	Methylsalicylate, an unknown substance boiling at 189°(760mm), and a little phenyletyl alcohol and citronellol.
5	65~70 (〃)	2.0	
6	72~76 (〃)	1.0	A little methylsalicylate, phenylethyl-alcohol, citronellol and their ester, also an unknown substance as in No. 4.
7	78~81 (〃)	0.8	Phenylethyl-alcohol, a little citronellol and their ester, and another unknown substance were found.
8	83~89 (〃)	0.5	Citronellol, a trace of geraniol and their ester and also an unknown substance as in No. 7.
9	90~96 (〃)	0.2	

As acid components, salicylic was isolated, the presence of phenylacetic,

and lower fatty acid such as butylic were only suspected by their odour.

From above results the presence of following compounds was ascertained in fermented tea leaves.

1. In the lower distillate, isovaler, isobutyl and butyl aldehyde.
2. In the middle destillate, β-γ hexenol and α-β hexenal.
3. In the higher distillate, methylsalicylate, phenylethylalcohol, citronellol and their ester.

Besides these, a trace of geraniol, and two unknown substances were noticed.

Study on the Essential Oil of Black Tea. Part II.—The Essential Oil of Formosan Black Tea. (pp. 639～643): By Ryo YAMAMOTO and Akiyosi KATO. (Agr. Chem. Lab., Taihoku Imp. University.)

In addition to the study of Part I, the flavour of fermented tea leaves after drying, namely the essential oil of "made black tea", has now been studied.

Two hundred thirty four kg of Formosan black tea of the standard quality were subjected to steam distillation at 112°. After concentrating the distillate acidic constituents were first separated by means of sodium hydroxide. From this part, which amounted to 5.7 g, methylsalicylate, another phenol of unknown constitution, salicylic and palmitic acids were isolated. The presence of butyric, isovaleranic, and phenylacetic acids were suggested.

The residual liquid was distillated in vacuum into the following fractions:

Distillate No.	Boiling point (4 mm)	Yield (g)	Distillate No	Boiling point (4 mm)	Yield (g)
1	30～55°	2.2	6	100～122°	1.8
2	55～68	3.0	7	123～140	1.9
3	69～74	1.2	8	140～151	1.5
4	77～88	3.2	Residue		5.9
5	88～95	1.8			

In this distillation, aldehydes were collected in a receiver cooled at −50°. But, they were, partly distilled away with ether when the original ethereal solution was concentrated. From the nature of 2·4 dinitrophenylhydrazone and the boiling point, it was supposed that a main part of aldehyde consisted of isovaler isobutyl, and butyl aldehyde and a little quantity of α-β hexenal. The total yield of aldehyde was 3.0 g. In the distillate, No. 1 isovaler aldehyde, an unidentified substance boiling at 110～124° under ordinary pressure, and α-β hexenal were found.

β-γ hexenol was isorated mainly from the distillate No. 2. This was separated as a phtalic ester and identified as 4′-Jod diphenylurethane.

An unknown substance of a strong odour, found in the distillate No. 1 was also met with in the distillates No. 2, No. 3 and No. 4. It constitutes an important flavour of black tea, but its chemical nature could not be determined. Phenylethylalcohol was isolated in the distillates No. 2 and No. 3. This was identified by converting it into phenylurethane.

Citronellol was found in the distillates No. 3, No. 4 and No. 5. Further, geraniol was isolated in these distillates. The latter was identified as its diphenylurethane, as well as by converting into citral by oxidation and subsequently naphtocinchonic acid.

These two compounds (citronellol and geraniol) seemed to have been existed both in free state and in ester form.

In the distillates No. 6 and No. 7 a liquid supposed to be an unsaturated terpene alcohol and an acid melting at 128° were isolated. These compounds existed provably in ester form and give a good flavour.

The essential oil of black tea in comparison with that of fermented tea leaf before drying, it can be said, in general, that there is no distinct difference between two. In the study of Part I, the presence of geraniol besides citronellol was suspected, and we have ascertained its presence in the present investigation. Furthermore the temperature of steam distillation was raised to 112° instead of 100°, so that the distillates above 100° under 4 mm pressure was now collected.

On the Amount of Solar Ultra Violet Rays. (Part. V)—On the weather type of the comming summer by the hypothesis of solar ultra violet rays (pp. 644～646): By Torataro HANZAWA.

Chemical Studies of Japanese Coccidae. IX.—On the Carbohydrates and Waxy Substances of Icerya purchasi Mask. (pp. 647～658): By Michio KAWANO and Ryunosuke MARUYAMA. (Research Lab., Osaka Factory of Sankyo Co.)

On the Peculiar Acid Soil. III. (pp. 659～673): By Shigeru OSUGI, Naohisa NISHIGAKI and Mitsuya YOSHIMI. (Agr. Chem. Lab., Kyoto Imp. University.)

On Systematic Study of Alcohol and Carbohydrate Oxidizing Bacteria isolated from Fruits, and a New Classification of the Oxidizing Bacteria (Continued.) (pp. 674~708): By Toshinobu ASAI.
(Agr. Chemical Loboratory, Tokyo Imp. University.)

Über den Synthese der α-Oxycaprinsäuren und α-Aminocaprinsäuren von oxidierendem Produkts der Ölsäuren. (S. 709 ~714): Von Yataro KOBATA (Biochemische Lab., Kyoto Kaiserliche Universität.)